SUPPORTING INFORMATION

Addition of a carbon fiber brush improves anaerobic digestion compared to external voltage application

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Nucleic acid extraction, library preparation, amplicon sequencing, and bioinformatics processing

The attached and suspended biomass samples were collected at the end of the third batch cycle from each reactor. DNA extraction was performed using the standard protocol for FastDNA Spin kit for Soil (MP Biomedicals, USA) with the following exceptions. 500 µL of sample, 480 µL Sodium Phosphate Buffer and 120 µL MT Buffer were added to a Lysing Matrix E tube. Bead beating was performed at 6 m/s for 4x40s (Albertsen et al., 2015). To validate the size and purity of the DNA extracts, gel electrophoresis using Tapestation 2200 and Genomic DNA screentapes (Agilent, USA) was performed. DNA concentration was measured using Qubit dsDNA HS/BR Assay kit (Thermo Fisher Scientific, USA). 16S rRNA gene region V4 sequencing libraries were prepared according to the Illumina protocol (Illumina, 2015), using up to 10 ng of extracted DNA as template, PCRBIO Ultra mix (PCR Biosystems, USA) and 400 nM of each forward and reverse tailed primer mix. PCR was conducted with the following program: Initial denaturation at 95°C for 2 min, 30 cycles of amplification (95°C for 15 s, 55°C for 15 s, 72°C for 50 s) and a final elongation at 72°C for 5 min. RNA was extracted using the standard protocol for RNeasy PowerMicrobiome Kit (Qiagen, Germany). Gel electrophoresis using Tapestation 2200 and RNA screentapes (Agilent, USA) was used for validation of product size and purity of a subset of RNA extracts. RNA concentration was measured using Qubit RNA HS/BR Assay kit (Thermo Fisher Scientific, USA). The extracted RNA was treated with the DNase Max kit (Qiagen, Germany) to completely remove DNA in the samples. The resulting RNA samples were quality controlled using RNA screentapes (Agilent, USA) and Qubit RNA HS/BR Assay kit (Thermo Fisher Scientific, USA). Using up to 10 ng of extracted RNA was used as template, PCR amplification was conducted with 2X Platinum

SuperFi RT-PCR Master Mix from the SuperScript IV One-Step RT-PCR System (Thermo Fisher Scientific, USA) and SuperScrip IV RT Mix (Thermo Fisher Scientific, USA). PCR was conducted with the following program: Reverse transcription at 60°C for 10 min, an initial denaturation at 98°C for 2 min, 25 cycles of amplification (98°C for 15 s, 55°C for 15 s, 72°C for 50 s) and a final elongation at 72°C for 5 min. For both DNA- and RNA-based libraries, duplicate PCR reactions were performed for each sample and the duplicates were pooled. The forward (515F) and reverse (806R) tailed primers were designed to target the 16S rRNA gene region V4 of both archaea and bacteria (Apprill et al., 2015). The primer tails enable attachment of Illumina Nextera adaptors necessary for sequencing in a subsequent PCR.

The resulting amplicon libraries were purified using the standard protocol for Agencourt Ampure XP Beads (Beckman Coulter, USA) with a bead to sample ratio of 4:5. DNA was eluted in 25 µL of nuclease free water (Qiagen, Germany). DNA concentration was measured using Qubit dsDNA HS Assay kit (Thermo Fisher Scientific, USA). Gel electrophoresis using Tapestation 2200 and D1000/High sensitivity D1000 screentapes (Agilent, USA) was used to validate product size and purity of a subset of sequencing libraries. Sequencing libraries were prepared from the purified amplicon libraries using a second PCR. Each PCR reaction (25 µL) contained PCRBIO HiFi buffer (1x), PCRBIO HiFi Polymerase (1 U/reaction) (PCRBiosystems, UK), adaptor mix (400 nM of each forward and reverse) and up to 10 ng of amplicon library template. PCR was conducted with the following program: Initial denaturation at 95°C for 2 min, 8 cycles of amplification (95°C for 20 s, 55°C for 30 s, 72°C for 60 s) and a final elongation at 72 ◦C for 5 min. The resulting sequencing libraries were purified and validated as mentioned above.

The purified sequencing libraries were pooled in equimolar concentrations and diluted to 2 nM. The samples were paired-end sequenced (2x300 bp) on a MiSeq (Illumina, USA) using a MiSeq Reagent kit v3 (Illumina, USA) following the standard guidelines for preparing and loading samples on the MiSeq. More than 10% PhiX control library was spiked in to overcome low complexity issues often observed with amplicon samples.

Forward and reverse reads were trimmed using Trimmomatic v. 0.32 (Bolger et al., 2014) with the settings SLIDINGWINDOW:5:3 and MINLEN: 225. The trimmed forward and reverse reads were merged using FLASH v. 1.2.7 (Magoč and Salzberg, 2011) with the settings -m 10 -M 250. The trimmed reads were dereplicated and formatted for use in the UPARSE workflow (Edgar, 2013). The dereplicated reads were clustered, using the usearch v. 7.0.1090 -cluster_otus command with default settings. Chimeras were also filtered by using this command. The OTU abundances were estimated using the usearch v. 7.0.1090 -usearch_global command with -id 0.97 -maxaccepts 0 -maxrejects 0. Taxonomy was assigned using the RDP classifier (Wang et al., 2007) as implemented in the parallel_assign_taxonomy_rdp.py script in QIIME (Caporaso et al., 2010), using confidence 0.8 and the SILVA database, release 132 (Quast et al., 2013). The results were analyzed in R v. 3.5.1 (R Core Team, 2017) through the Rstudio IDE using the ampvis package v.2.5.8 (Albertsen et al., 2015).

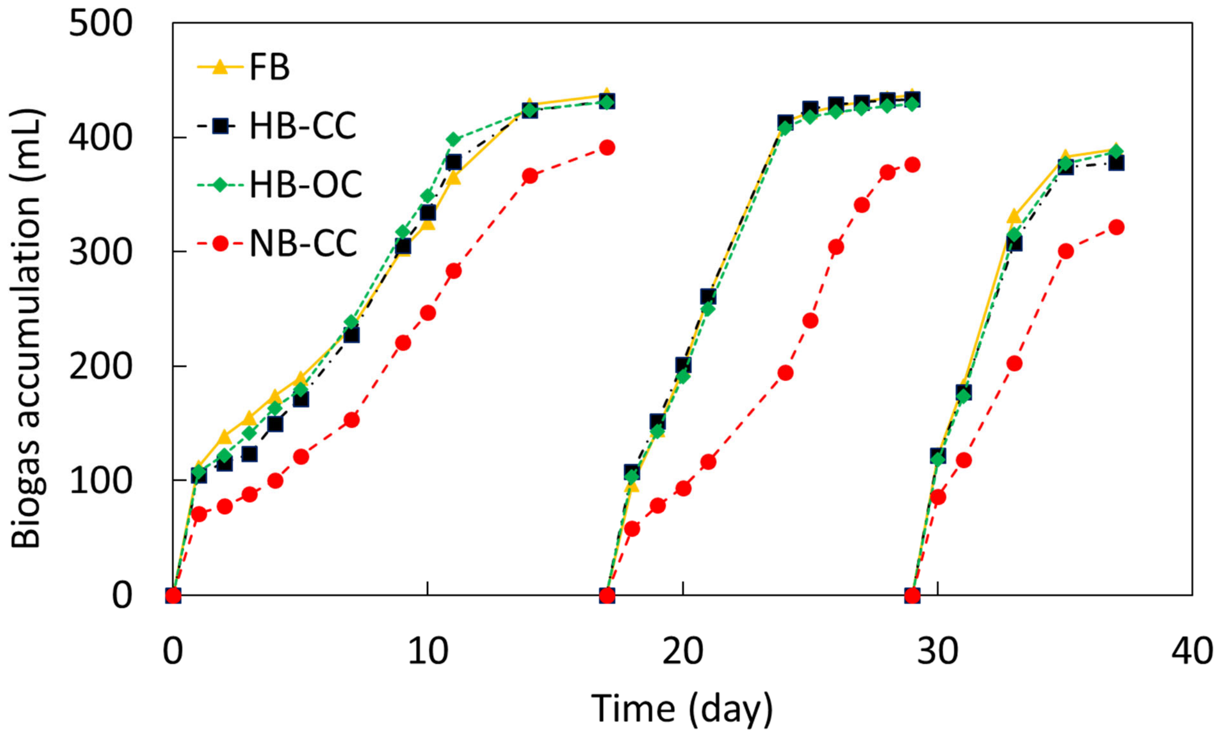

Fig. S1. Biogas accumulation during three successive batch cycles. For gas accumulation data of HB-CC during the second and third cycles, only one of the duplicates was reported due to substantial gas leakage from the other reactor.

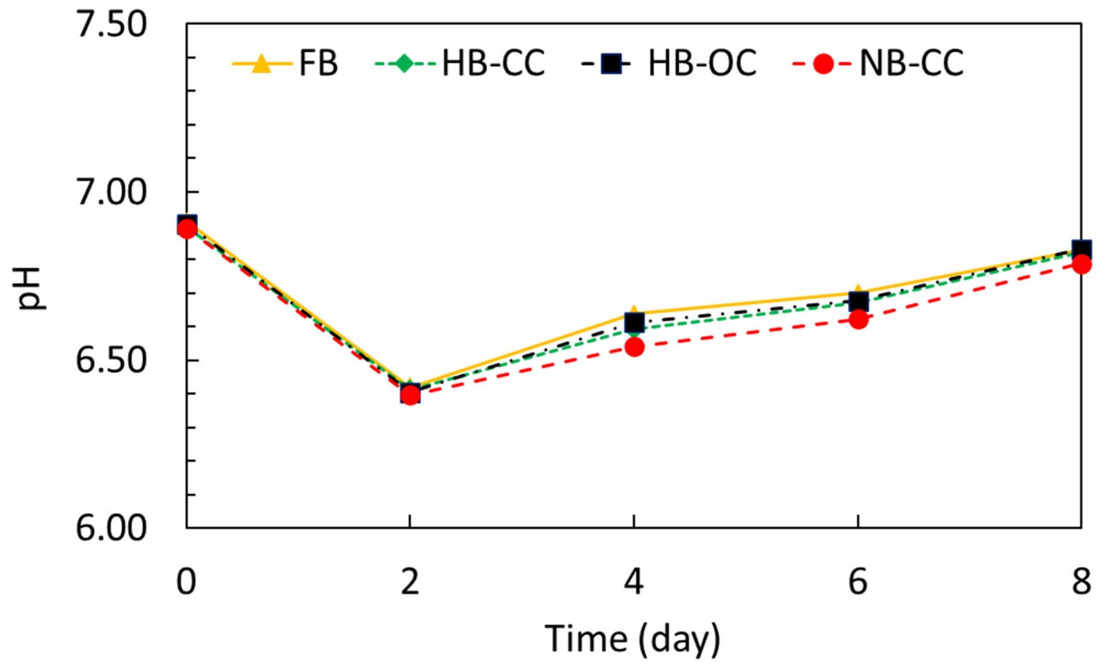

Fig. S2. pH change during the third batch cycle.

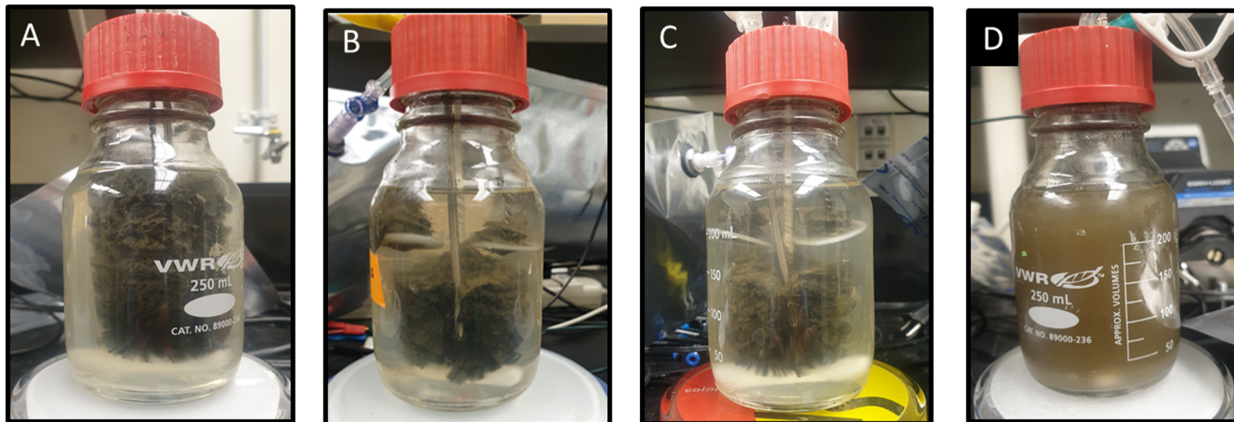

Fig. S3. Photographs of (A) FB, (B) HB-CC, (C) HB-OC and (D) NB-CC during the third batch operation. Compared to the NB-CC reactor, much clearer mixed liquors were observed in other reactors that had greater biomass on the brushes.

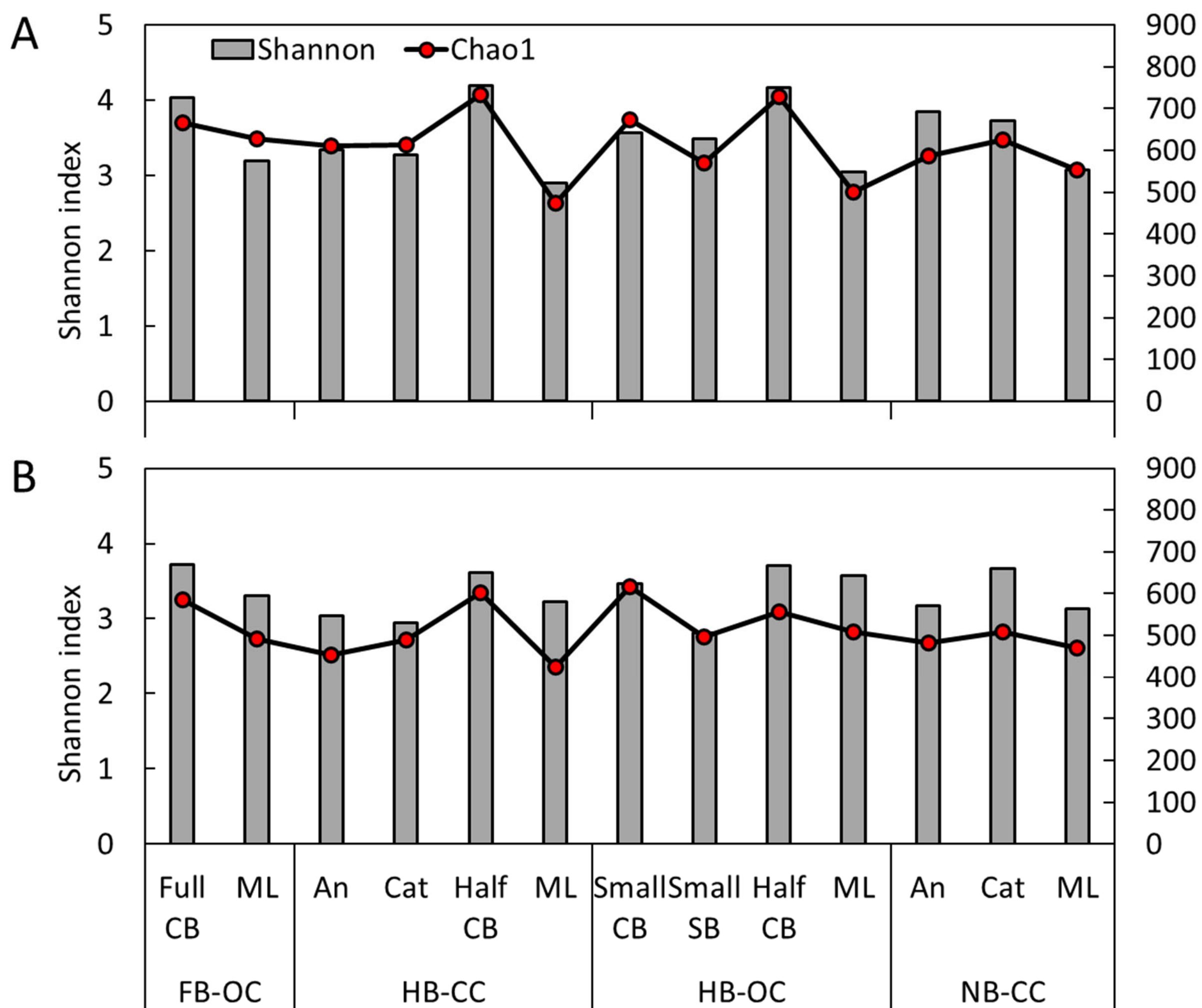

Fig. S4. The alpha diversity using Shannon and Chao1 indices based on (A) 16S rDNA- and (B) 16S rRNA-libraries (CB; carbon brush, ML; mixed liquor, An; anode, Cat; cathode, SB; stainless steel brush).

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